Esp

Week 14

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

1 Digestion of pSB1K3-T7-EB and ligation into the pSB1C3 backbone

Responsible

Shuangjia Xue and Reskandi Chastelia Rudjito

Protocols used

3A Assembly (Digestion and Ligation)

Modifications and comments to protocols

For digestion, the total volume for each reaction was 10 μl. 300 ng DNA was digested with 0.3 unit of restriction enzyme. Both EB and backbone were double digested with EcoRI and SpeI. For ligation, due to the low concentration of digested EB, the proportion for insert to backbone was 3:1.

Experimental Set Up

Table 1: Setup for digestion of pSB1C3 backbone

Reagents	Volume [μl]	Comments
pSB1C3 backbone	7.7	300 ng
Buffer SH 10X	1	1X
EcoRI	0.3	0.3 unit
SpeI	0.3	0.3 unit
Sterile water	0.7	adjust to 10 μl total volume

Table 2: Setup for digestion of pSB1K3-T7-EB

Reagents	Volume [μl]	Comments
pSB1K3-T7-EB	3.8	300 ng
Buffer SH 10X	1	1X
EcoRI	0.3	0.3 unit
SpeI	0.3	0.3 unit
Sterile water	4.6	adjust to 10 μl total volume

Table 3: Setup for ligation

Reagents	Volume [μl]
Digested T7-EB	25.2
Digested backbone	10.2
T4 ligase	0.7
10x T4 DNA ligase cutsmart buffer	4

Results and Conclusions

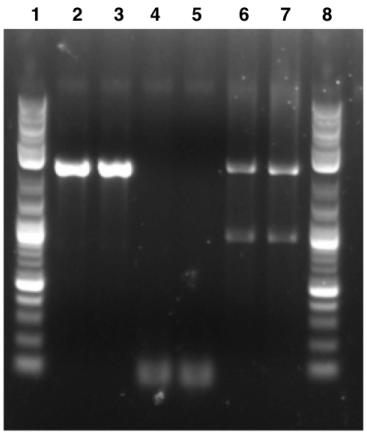

Figure 1: Result of the digestion product. (1) DNA ladder (2) digested backbone (3) digested backbone 2 (4-5) irrelevant samples (6) Digested EB1 (7) Digested EB2 (8) DNA ladder

As shown in the Figure 1, the double digestion was successful. Two clear bands occur with correct sizes. So we continued with gel extraction and ligation overnight.

Discussion and Troubleshooting

Transformation of ligation product.